SITA
Società Italiana di Terapia Antinfettiva
Antibatterica Antivirale Antifungina

14° CONGRESSO NAZIONALE

GENOVA | 21-22 novembre 2024

SARS-CoV-2 viral evolution:
immune escape, transmission, pathogenicity

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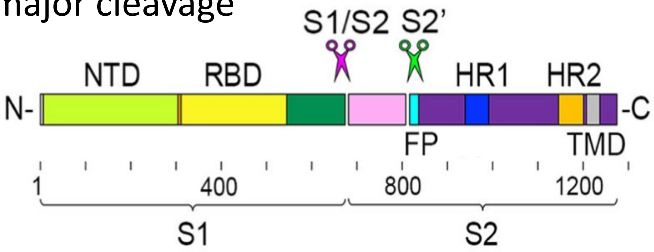
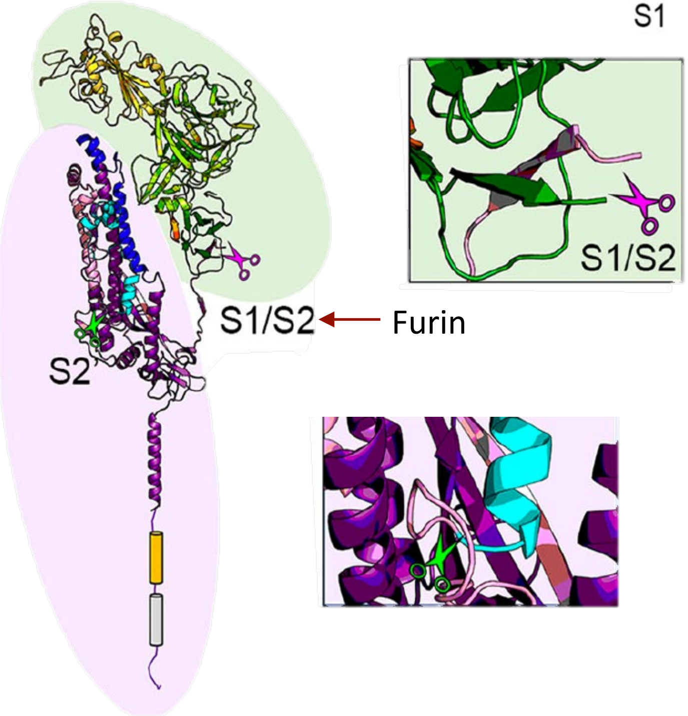
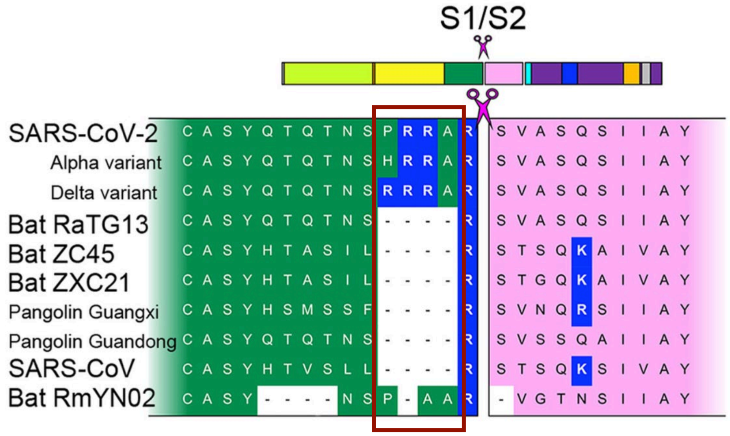
The Spike protein has two major cleavage sites:

- S1/S2 (685/686)
- S2’ (815/816)





Furin



SARS-CoV-2	C A S Y Q T Q T N S P R R A R	S V A S Q S I I A Y
Alpha variant	C A S Y Q T Q T N S H R R A R	S V A S Q S I I A Y
Delta variant	C A S Y Q T Q T N S R R R A R	S V A S Q S I I A Y
Bat RaTG13	C A S Y Q T Q T N S - - - - R	S V A S Q S I I A Y
Bat ZC45	C A S Y H T A S I L - - - - R	S T S Q K A I V A Y
Bat ZXC21	C A S Y H T A S I L - - - - R	S T G Q K A I V A Y
Pangolin Guangxi	C A S Y H S M S S F - - - - R	S V N Q R S I I A Y
Pangolin Guandong	C A S Y Q T Q T N S - - - - R	S V S S Q A I I A Y
SARS-CoV	C A S Y H T V S L L - - - - R	S T S Q K S I V A Y
Bat RmYN02	C A S Y - - - - N S P - A A R	- V G T N S I I A Y

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Alpha    HCoV-NL63   735 - GICADGSLI----PVRPRNSS - 751
Alpha    HCoV-229E   554 - GVCADGSII----AVQPRNVS - 570
Beta 2a  HCoV-OC43   753 - GYCVDYSK-----NRRSRGAI - 768
Beta 2a  HCoV-HKU1   742 - GFCVDYNSPSSSSRRKRRSI - 762
Beta 2b  SARS-CoV    655 - GICASYHTVS-L----LRSTS - 670
Beta 2b  SARS-CoV-2  669 - GICASYQTQT-NSPRRARSVA - 688
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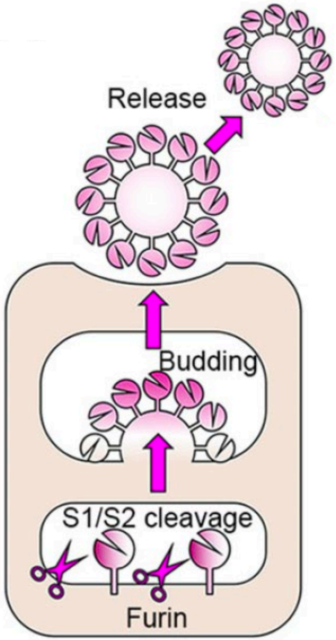
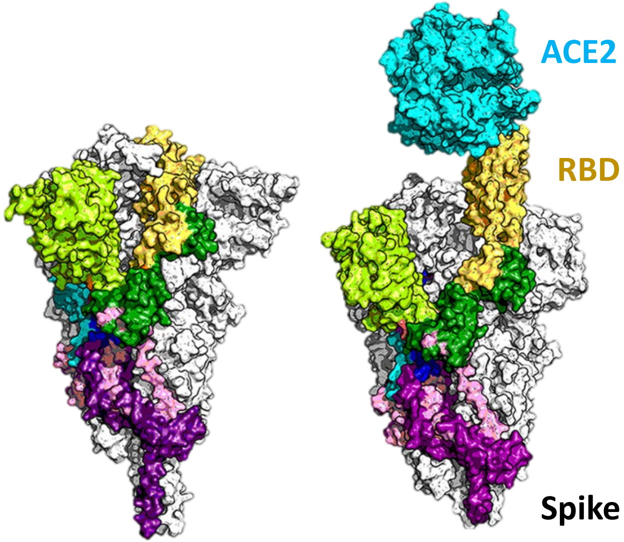
Takeda, Microbiology and immunology, 2021

Spike conformation for ACE2 interaction



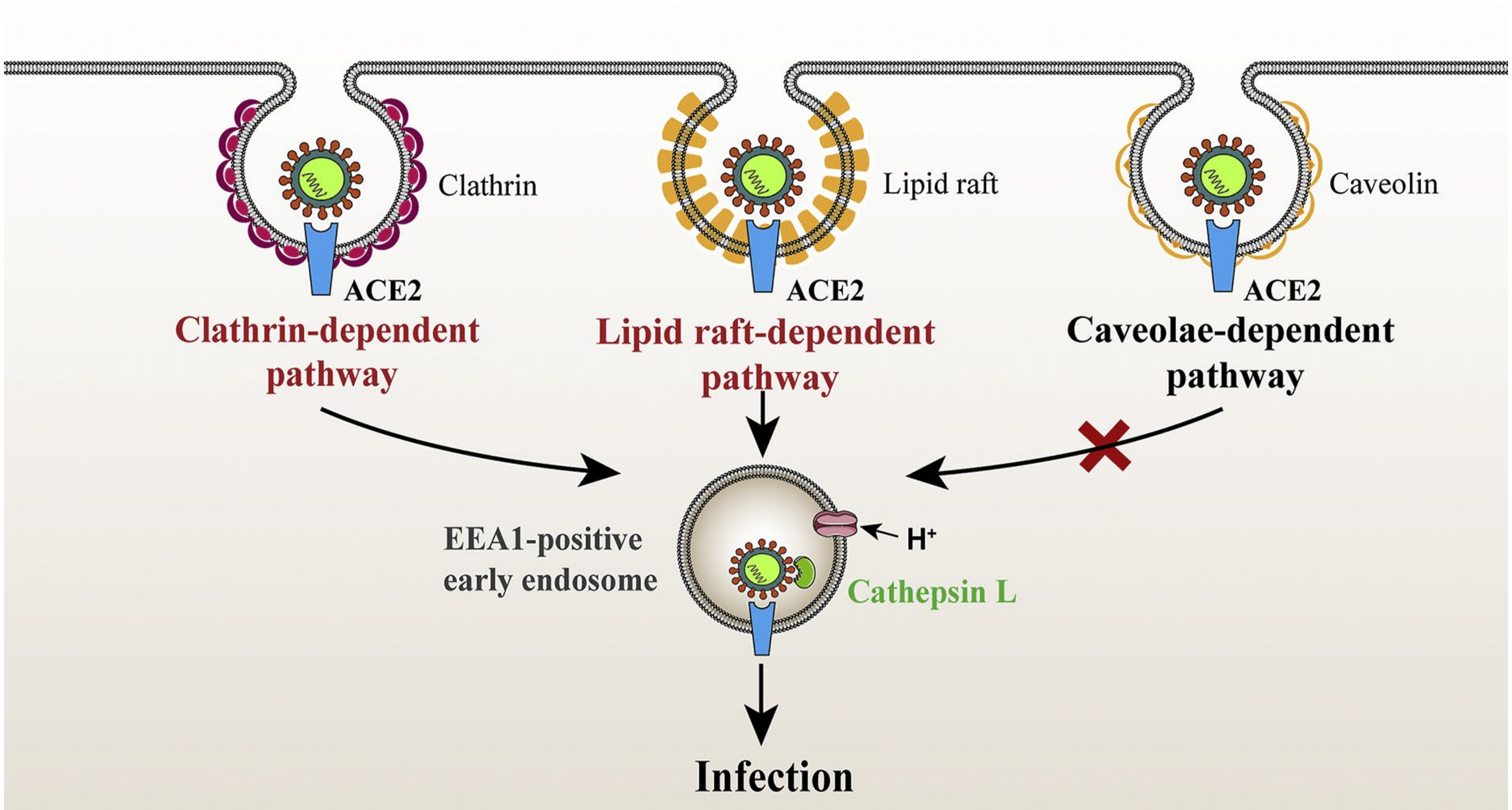


Down state

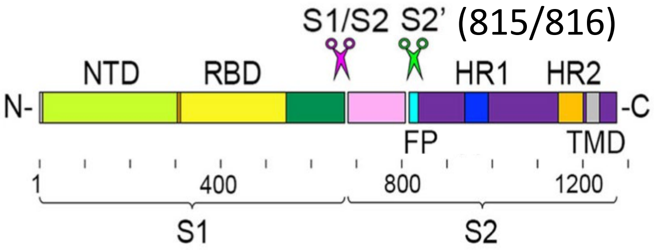
Up state

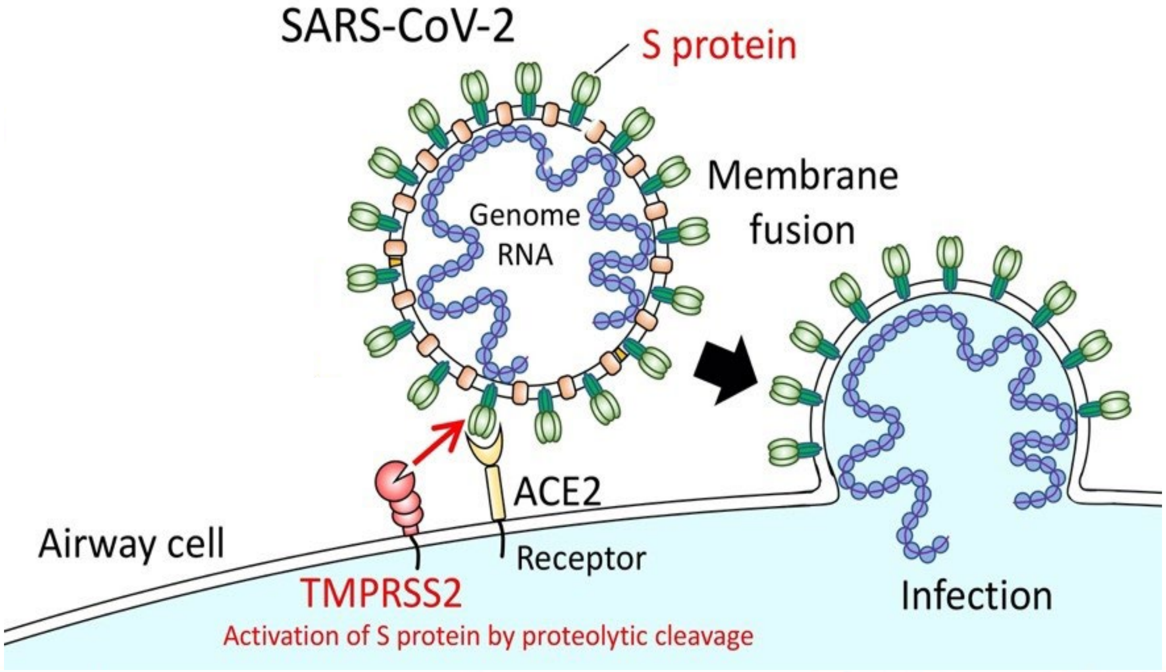
Takeda, Microbiology and immunology, 2021

ACE2-mediated SARS-CoV-2 endocytosis

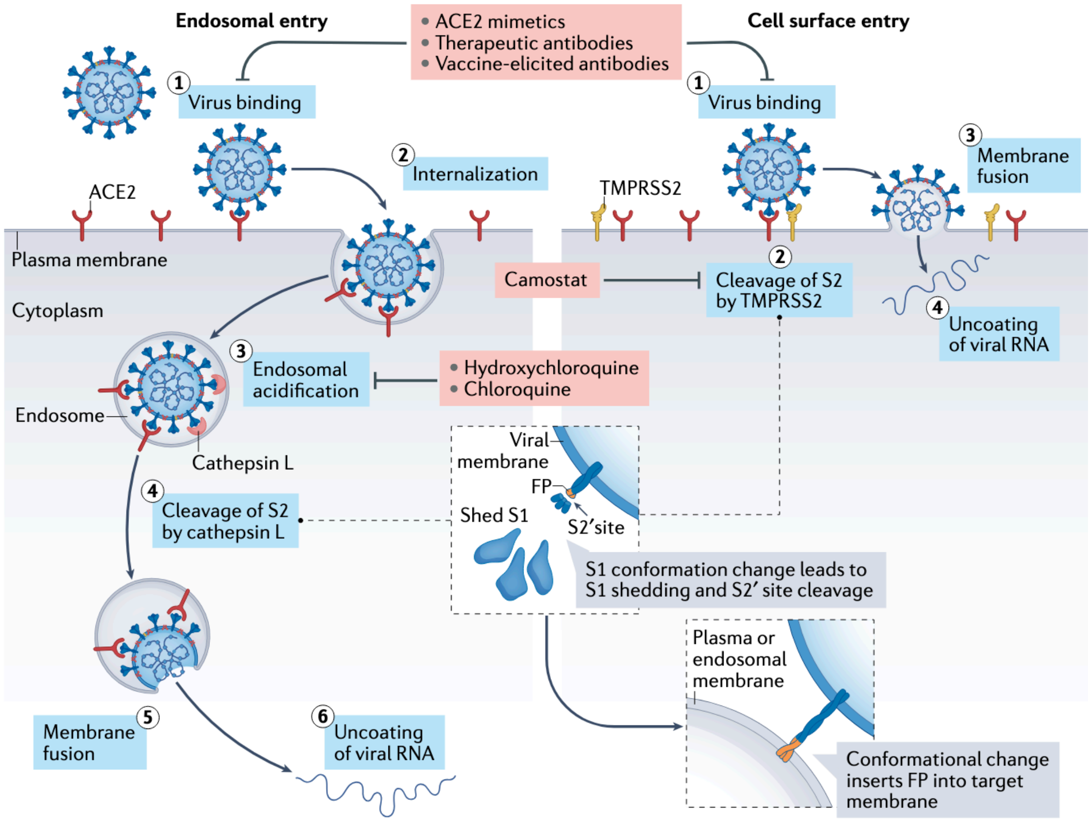


ACE2 and TMPRSS2-mediated SARS-CoV-2 fusion

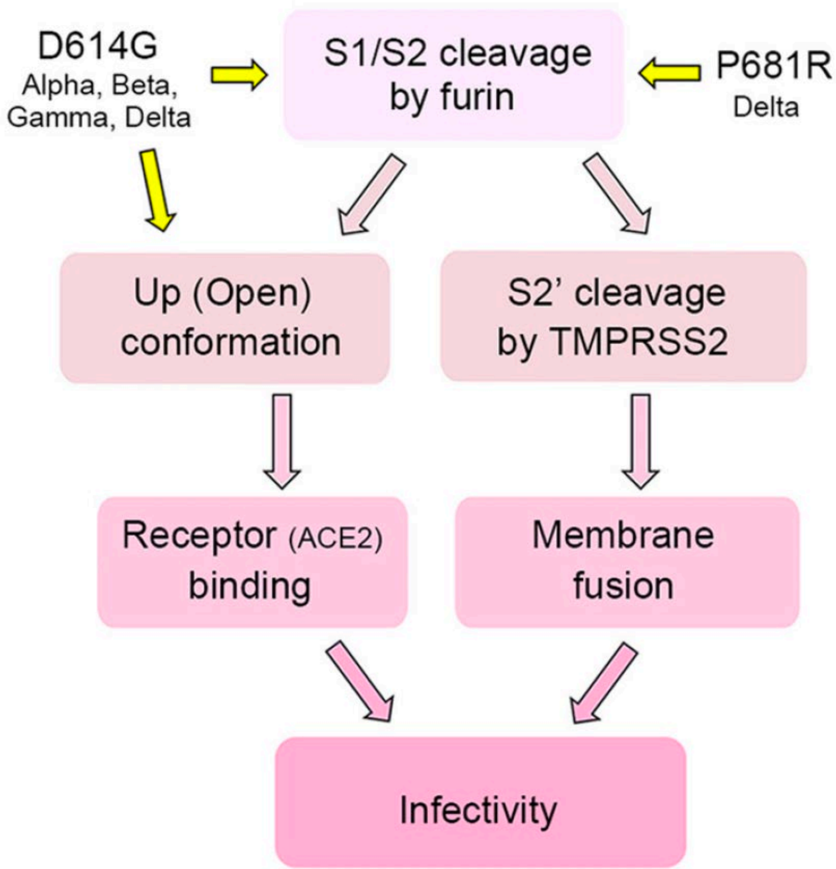




Entry pathways for SARS-CoV-2



Jackson et al, Nat Rev, 2022



D614G
Alpha, Beta, Gamma, Delta

S1/S2 cleavage by furin

P681R
Delta

Up (Open) conformation

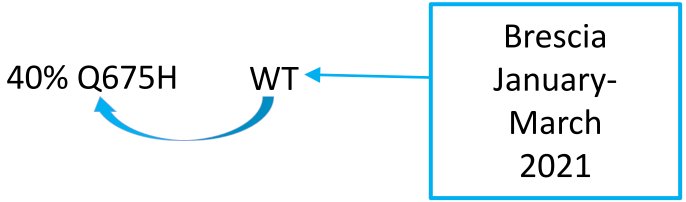
S2’ cleavage by TMPRSS2

Receptor (ACE2) binding

Membrane fusion

Infectivity

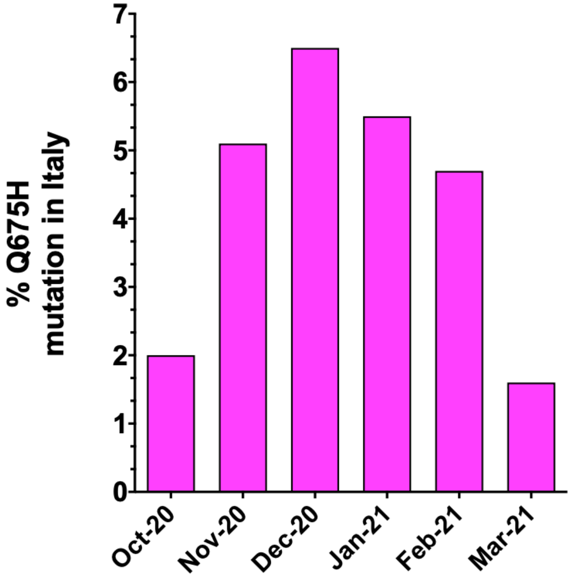
Reporting of SARS-CoV-2 Q675H mutation

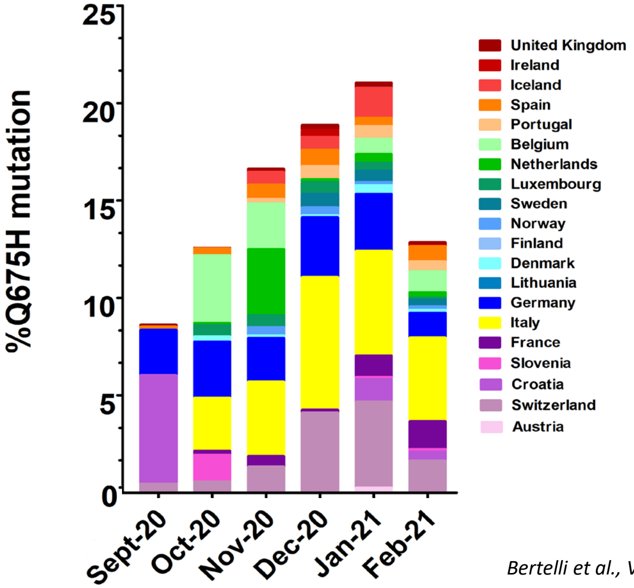


40% Q675H

WT

Brescia
January-
March
2021

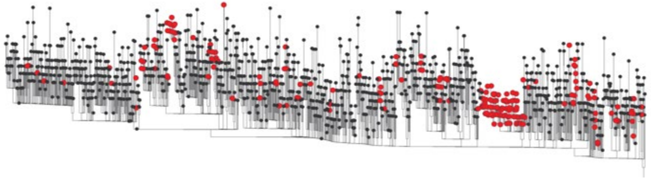




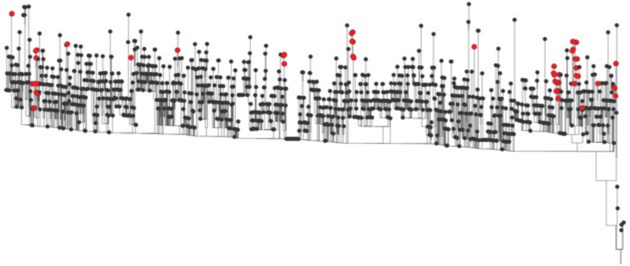
Bertelli et al., Viruses, 2021

Phylogenetic analysis shows that Q675H mutation arises by homoplasy events

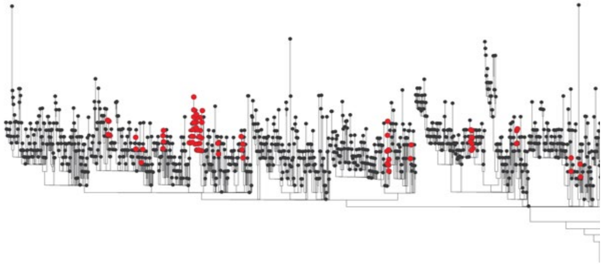
B.1.1.7



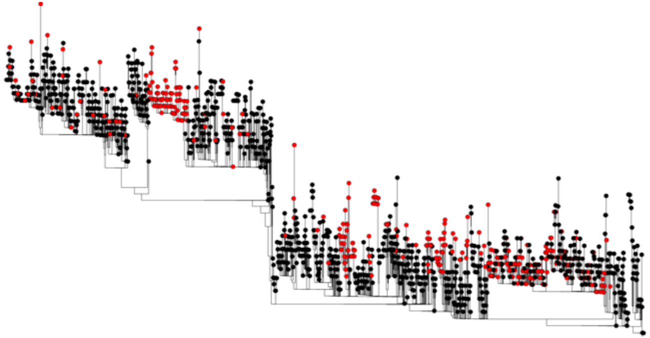
P.1



B.1.351

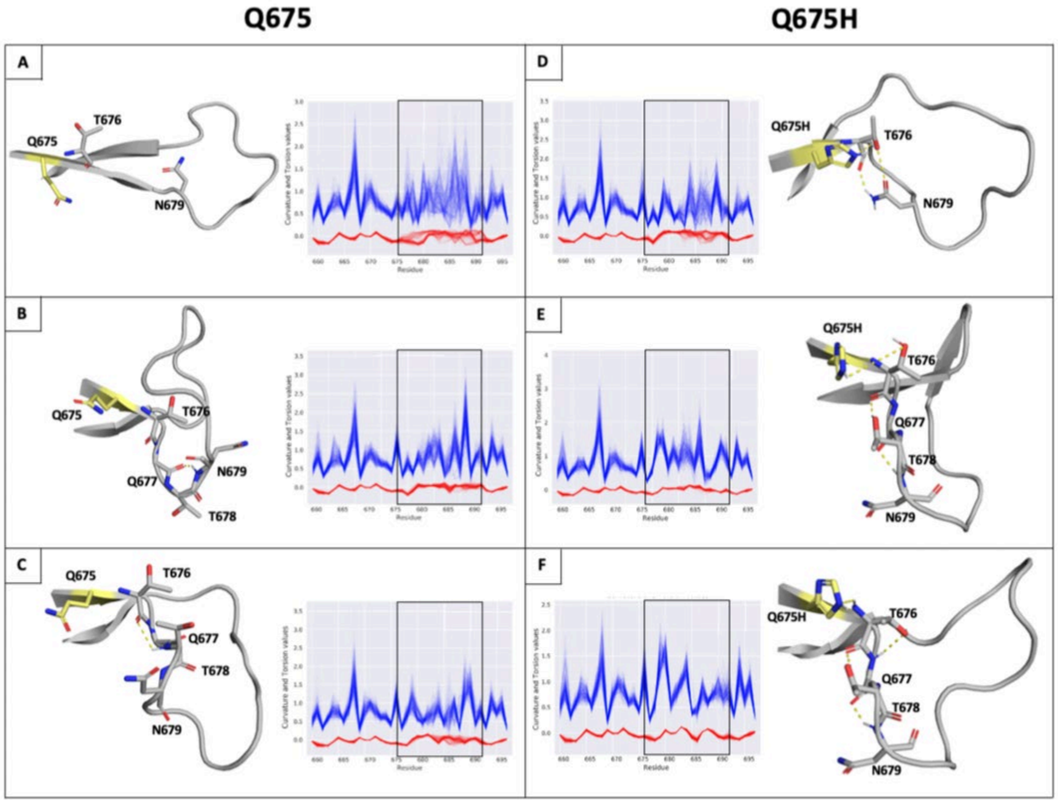


B.1.617.2



Bertelli et al., Viruses, 2021

Effects of Q675H on conformation of the furin binding motif



GLN to HIS mutation showed a positive (ΔΔG value is 0.665Kcal Kcal/mol) and a negative (ΔΔSVibENCoM value is –0.108 kcal.mol-1K-1) change in vibrational entropy energy between wild-type and mutant proteins.

Bertelli et al., Viruses, 2021

Cell

Article

Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus

Korber B et al, 2020

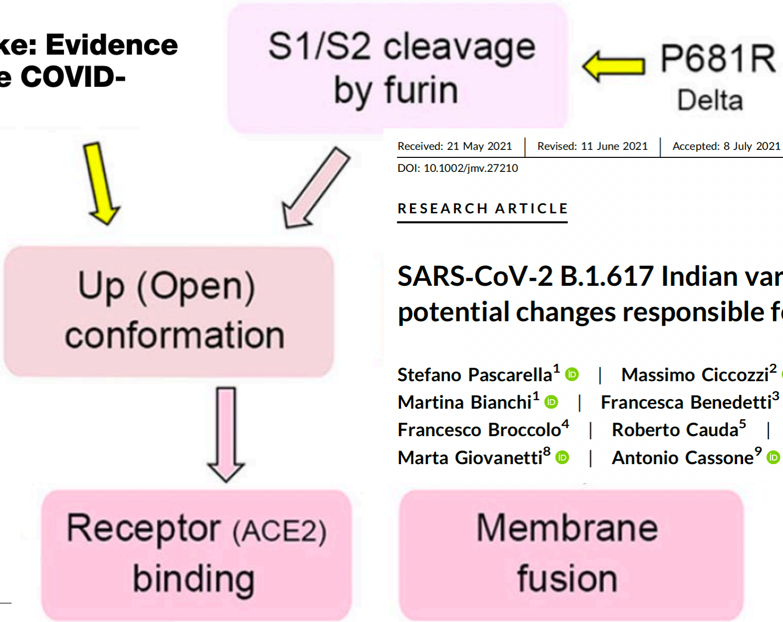
CORONAVIRUS

Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England

Davies *et al.*, *Science* **372**, eabg3055 (2021)   9 April 2021

SARS-CoV-2 B.1.1.7 and B.1.351 spike variants bind human ACE2 with increased affinity

Ramanathan M et al, Lancet Infect Dis, 2021



S1/S2 cleavage by furin

P681R

Delta

Up (Open) conformation

Receptor (ACE2) binding

Membrane fusion

Received: 21 May 2021 | Revised: 11 June 2021 | Accepted: 8 July 2021

DOI: 10.1002/jmv.27210

JOURNAL OF MEDICAL VIROLOGY WILEY

RESEARCH ARTICLE

SARS-CoV-2 B.1.617 Indian variants: Are electrostatic potential changes responsible for a higher transmission rate?

Stefano Pascarella[1] | Massimo Ciccozzi[2] | Davide Zella[3] |
Martina Bianchi[1] | Francesca Benedetti[3] | Domenico Benvenuto[2] |
Francesco Broccolo[4] | Roberto Cauda[5] | Arnaldo Caruso[6] | Silvia Angeletti[7] |
Marta Giovanetti[8] | Antonio Cassone[9]

Received: 28 January 2021 | Revised: 1 March 2021 | Accepted: 4 March 2021

DOI: 10.1002/jcp.30367

Journal of Cellular Physiology WILEY

RESEARCH ARTICLE

Higher infectivity of the SARS-CoV-2 new variants is associated with K417N/T, E484K, and N501Y mutants: An insight from structural data

Abbas Khan[1] | Tauqir Zia[2] | Muhammad Suleman[3] | Taimoor Khan[1] |
Syed Shujait Ali[3] | Aamir Ali Abbasi[4] | Anwar Mohammad[5] | Dong-Qing Wei[1,6,7]

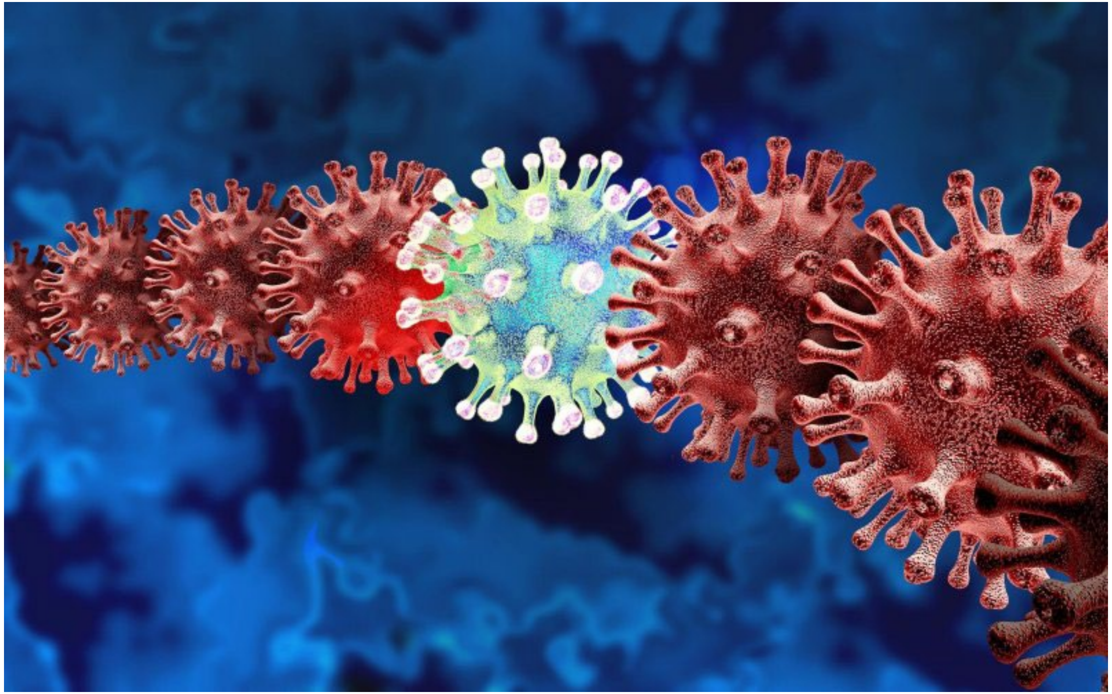
International Journal of *Molecular Sciences*
https://www.mdpi.com/journal/ijms
MDPI

Article

Improved Binding Affinity of Omicron's Spike Protein for the Human Angiotensin-Converting Enzyme 2 Receptor Is the Key behind Its Increased Virulence

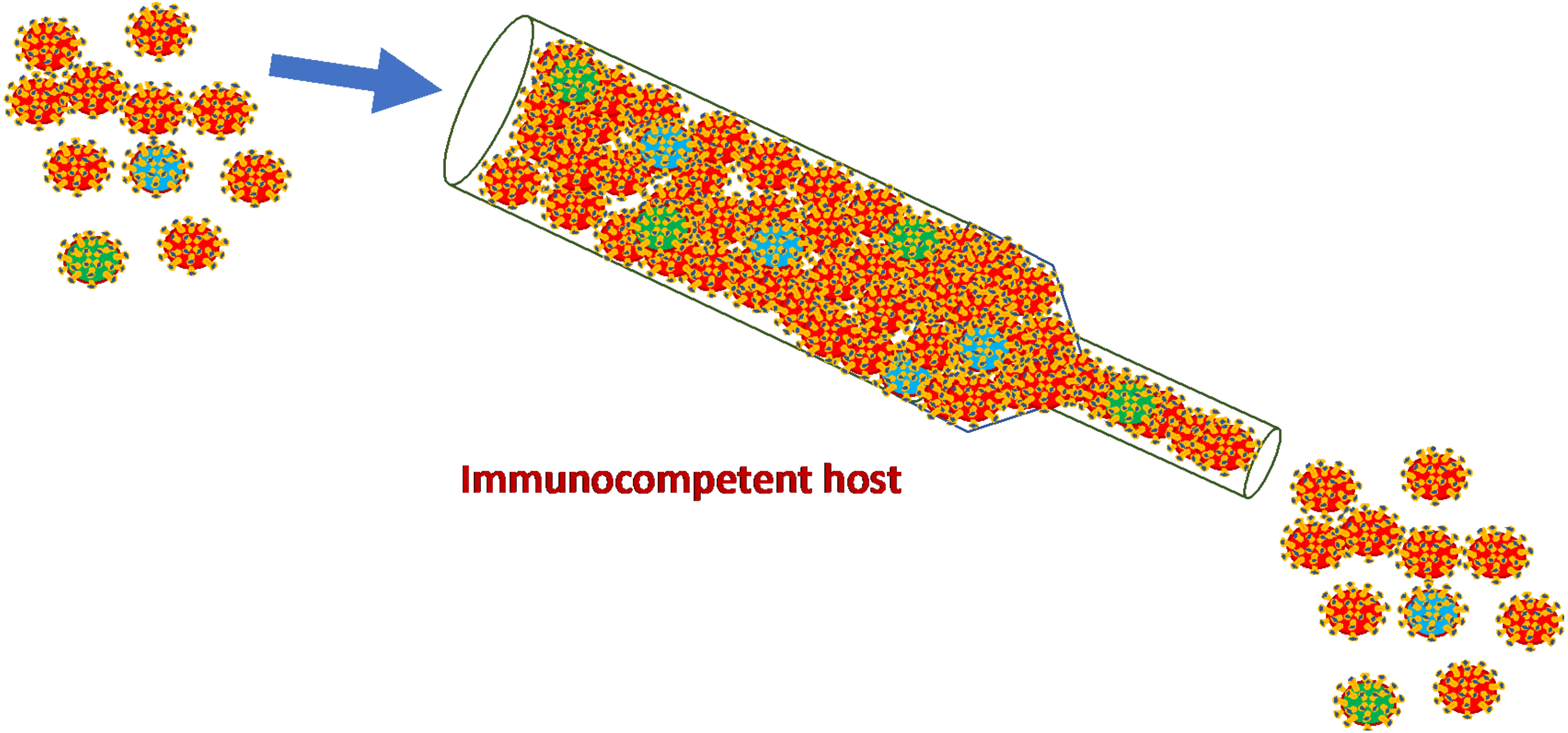
Rajender Kumar [1], Natarajan Arul Murugan [2,*,†] and Vaibhav Srivastava [1,*]

Viral evolution is a long game: How does it occur?

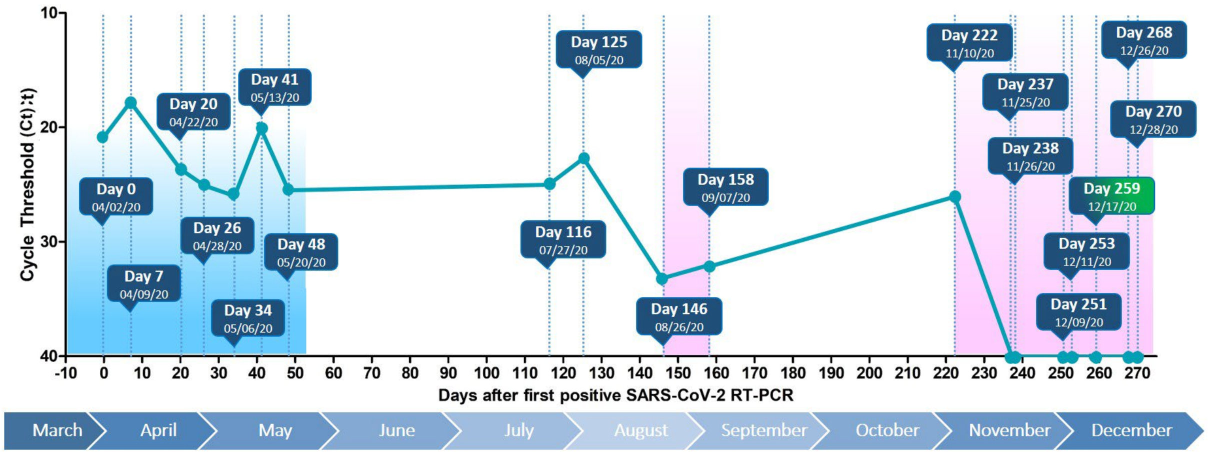


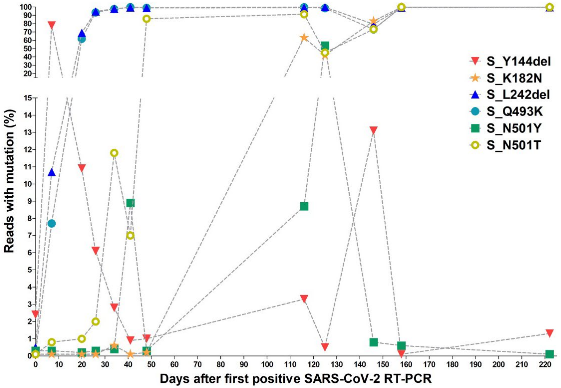
The Intra-host evolution

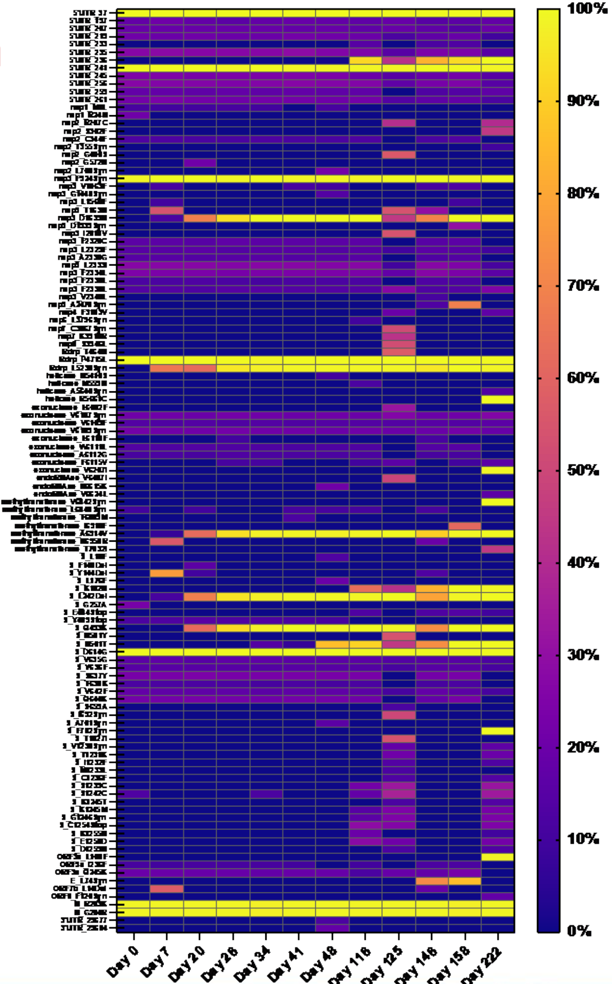
The bottleneck-mediated quasispecies restriction during spread of virions



SARS-CoV-2 intra-host evolution



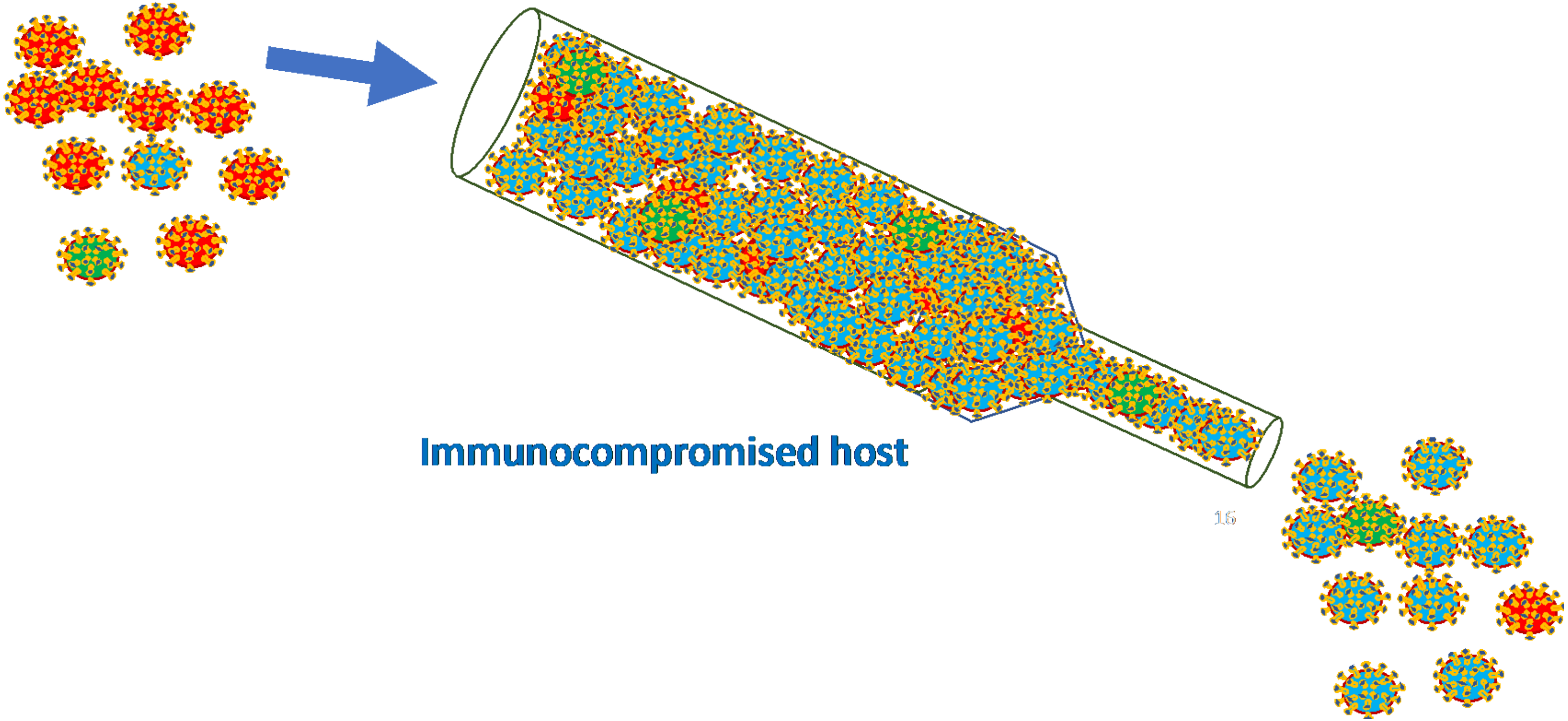




Fiorentini et al., Lancet Infect Dis, 2021

Caccuri et al., Virus Evol, 2022

The bottleneck-mediated quasispecies restriction during spread of virions

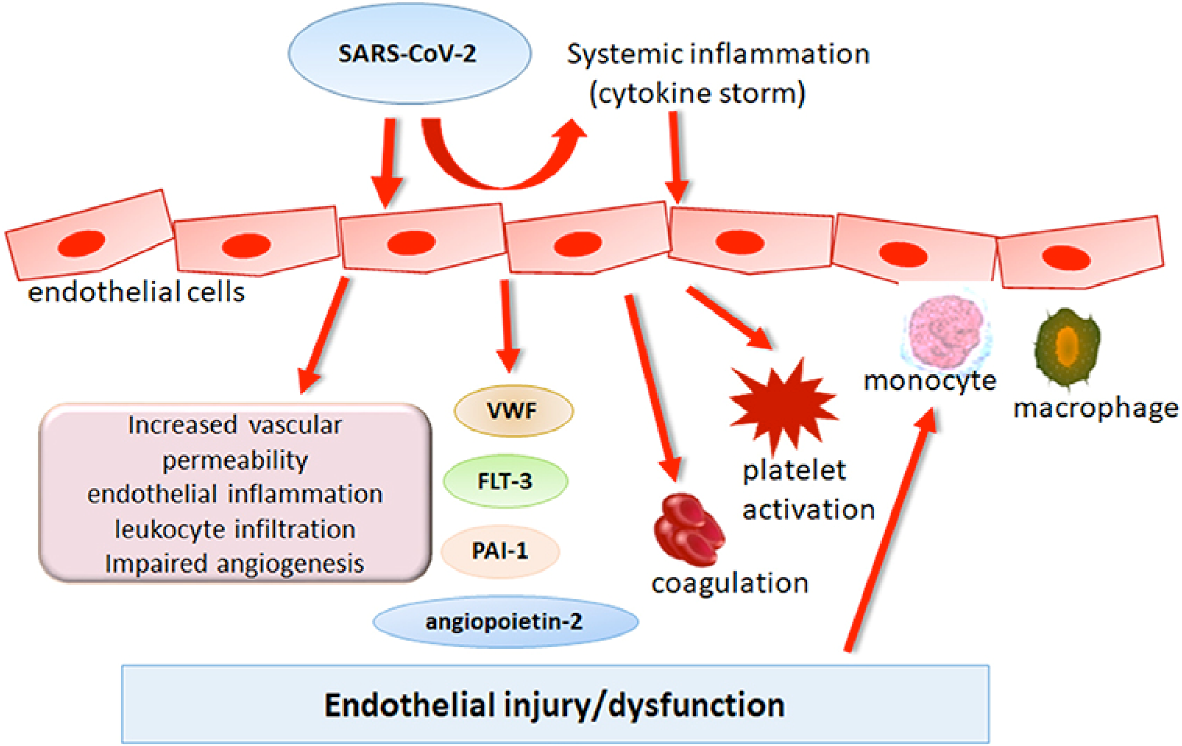


Conclusion I

- SARS-CoV-2 possesses two evolutionary sites
  - The first is represented by RBD domain which is directly involved in ACE2 binding. Its evolution enhances viral entry
  - The second one is represented by the furin cleavage site. Its evolution increases viral adaptation to the human host
  - Both the sites contribute to a better global viral fitness

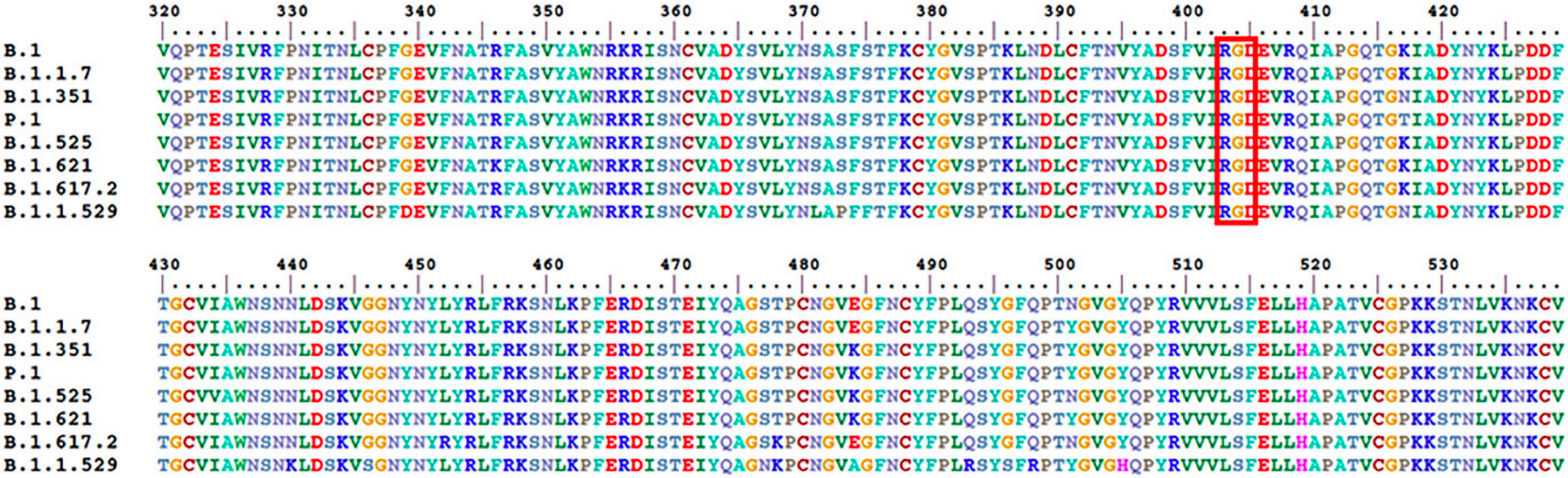
- SARS-CoV-2 evolution occurs in immunocompromised human hosts

Role of SARS-CoV-2 in endothelial dysregulation



Adapted by Liu and Zhang, Front Med, 2021

Changes in the Receptor Binding Domain (RBD) of SARS-CoV-2 VOCs



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           320       330       340       350       360       370       380       390       400       410       420
           |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....
B.1        VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDF
B.1.1.7    VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDF
B.1.351    VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDF
P.1        VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGTIADYNYKLPDDF
B.1.525    VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDF
B.1.621    VQPTESIVRFPNITNLCPFGEVFNATKFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDF
B.1.617.2  VQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDF
B.1.1.529  VQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNLAPFFTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDF

           430       440       450       460       470       480       490       500       510       520       530
           |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....
B.1        TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
B.1.1.7    TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
B.1.351    TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
P.1        TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
B.1.525    TGCVVAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
B.1.621    TGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
B.1.617.2  TGCVIAWNSNNLDSKVGGNYNYRYRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
B.1.1.529  TGCVIAWNSNKLDSKVSGNYNYLYRLFRKSNLKPFERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPTYGVGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV
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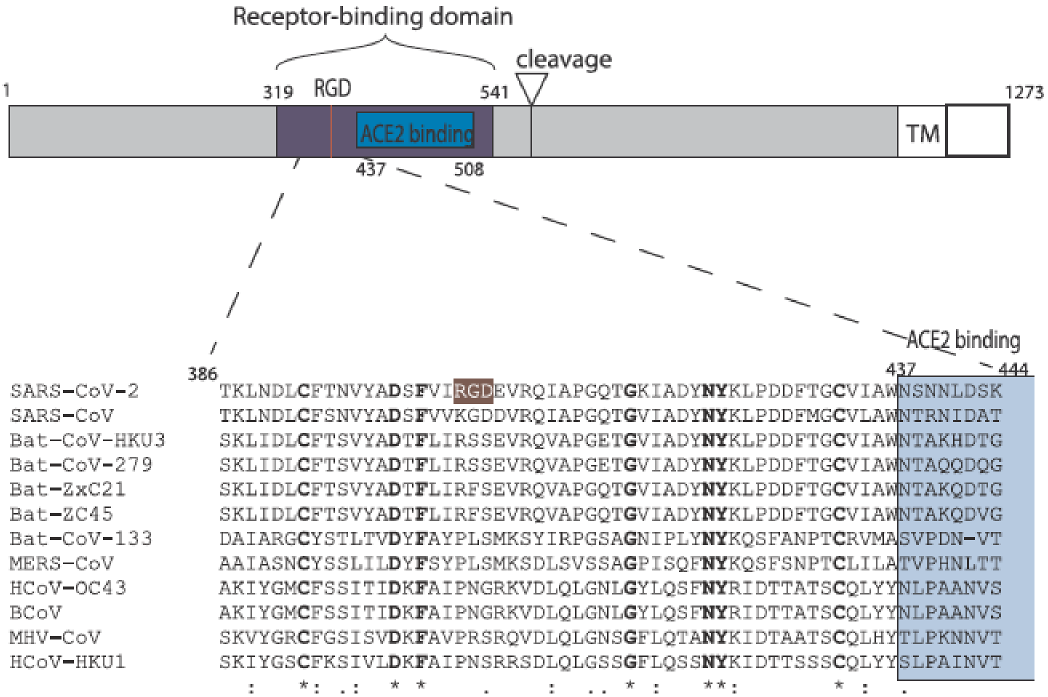

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SARS-CoV-2      TKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSK
SARS-CoV        TKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDAT
Bat-CoV-HKU3    SKLIDLCFTSVYADTFLIRSSEVRQVAPGETGVIADYNYKLPDDFTGCVIAWNTAKHDTG
Bat-CoV-279     SKLIDLCFTSVYADTFLIRSSEVRQVAPGETGVIADYNYKLPDDFTGCVIAWNTAQQDQG
Bat-ZxC21       SKLIDLCFTSVYADTFLIRFSEVRQVAPGQTGVIADYNYKLPDDFTGCVIAWNTAKQDTG
Bat-ZC45        SKLIDLCFTSVYADTFLIRFSEVRQVAPGQTGVIADYNYKLPDDFTGCVIAWNTAKQDVG
Bat-CoV-133     DAIARGCYSTLTVDYFAYPLSMKSYIRPGSAGNIPLYNYKQSFANPTCRVMASVPDN-VT
MERS-CoV        AAIASNCYSSLILDYFSYPLSMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTT
HCoV-OC43       AKIYGMCFSSITIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANVS
BCoV            AKIYGMCFSSITIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANVS
MHV-CoV         SKVYGRCFGSISVDKFAVPRSRQVDLQLGNSGFLQTANYKIDTAATSCQLHYTLPKNNVT
HCoV-HKU1       SKIYGSCFKSIVLDKFAIPNSRRSDLQLGSSGFLQSSNYKIDTTSSSCQLYYSLPAINVT
                  :   *: .:  * *    .    :  .. * :   **:       * :  .
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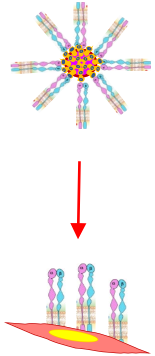
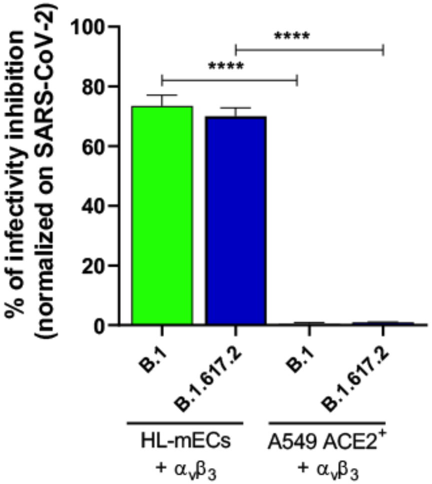
Fig. 2. Schematic representation of SARS-CoV-2 S-protein with a focus on the receptor-binding domain. The sequences of 12 betacoronavirus were aligned using MAFFT (Katoh et al., 2019). The receptor-binding domain and the ACE2 receptor-binding region are colored in blue and light blue, respectively. The RGD motif of SARS-CoV-2 is highlighted in color. Numbers refer to the SARS-CoV-2 spike protein sequence.

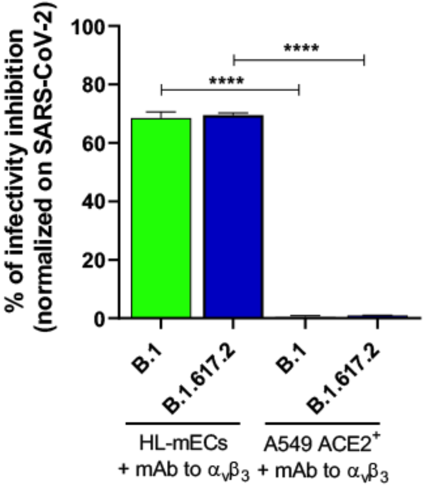
$\alpha_v\beta_3$ integrin inihibits SARS-CoV-2 entry into HL-mECs

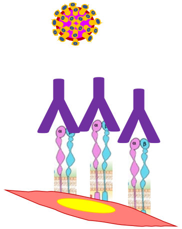
Virus + integrin $\alpha_v\beta_3$
were used to infect HL-mECs

HL-mECs were pre-treated with
mAb against $\alpha_v\beta_3$
and then infected with SARS-CoV-2



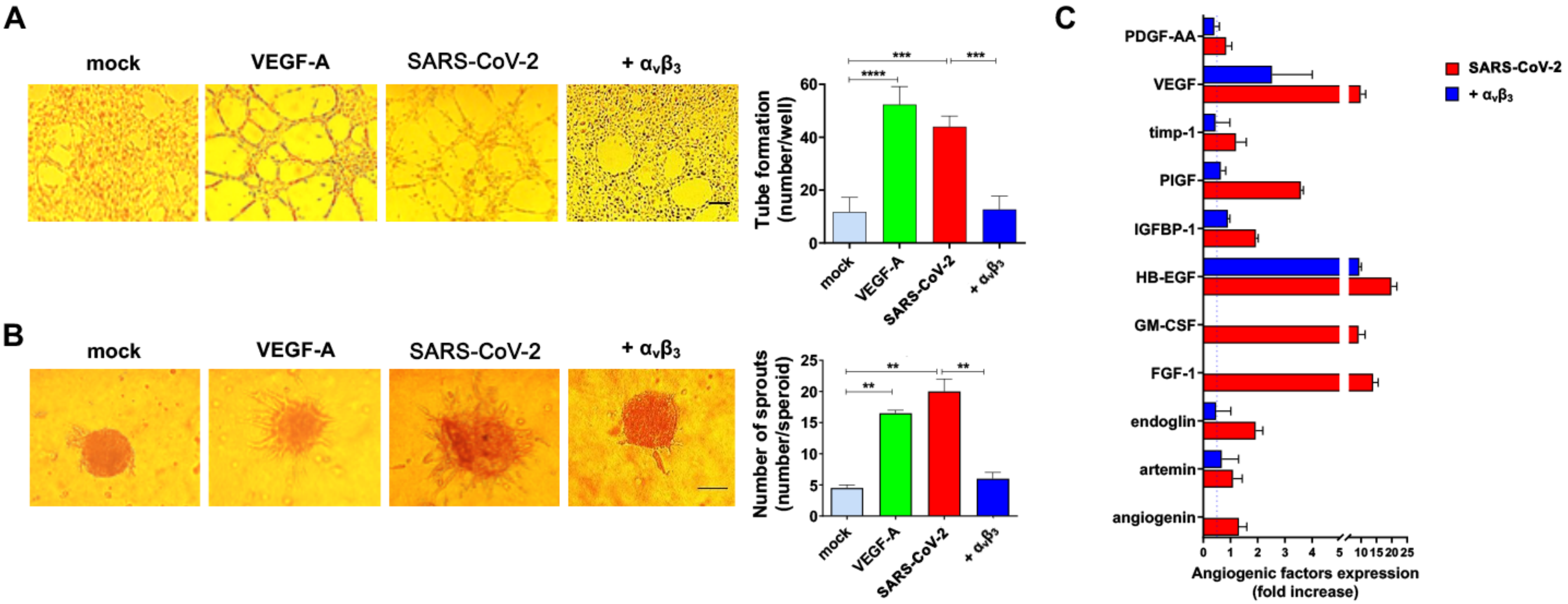




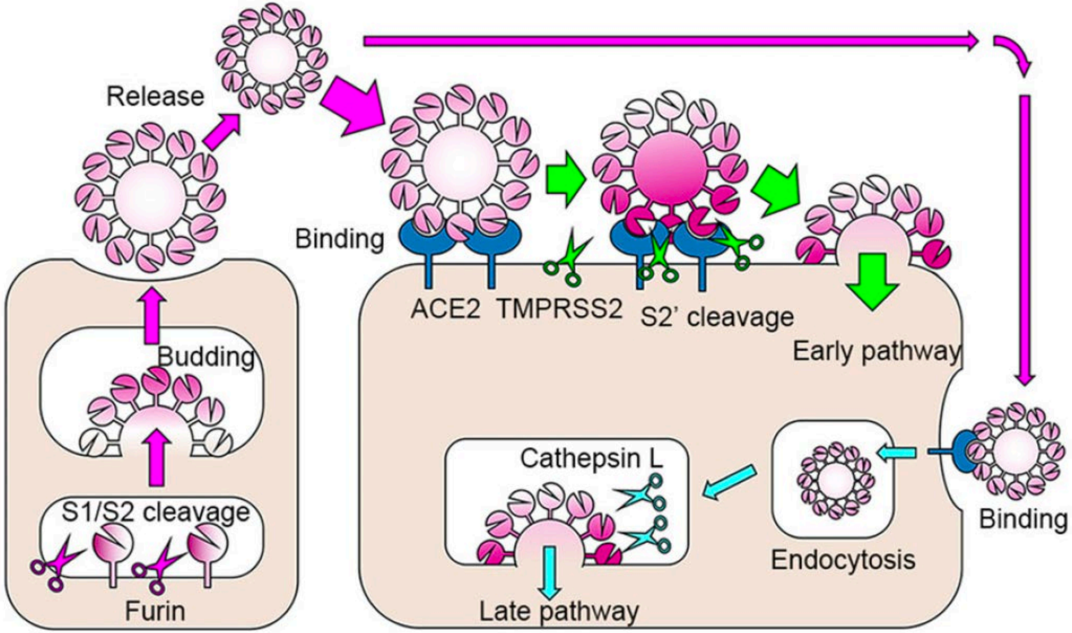


Bugatti et al, Viruses, 2022

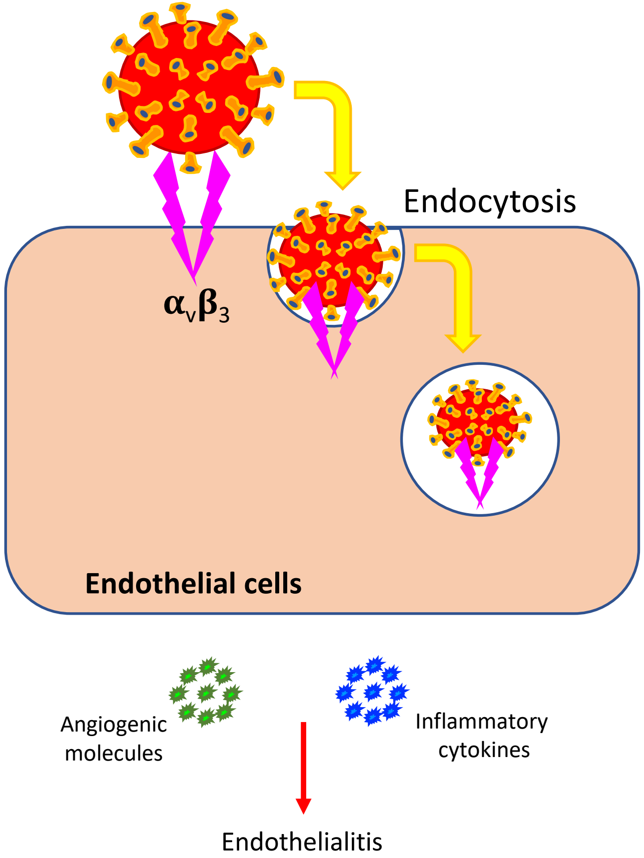
$\alpha_v\beta_3$ counteracts SARS-CoV-2 proangiogenic effects



Bugatti et al, Viruses, 2022



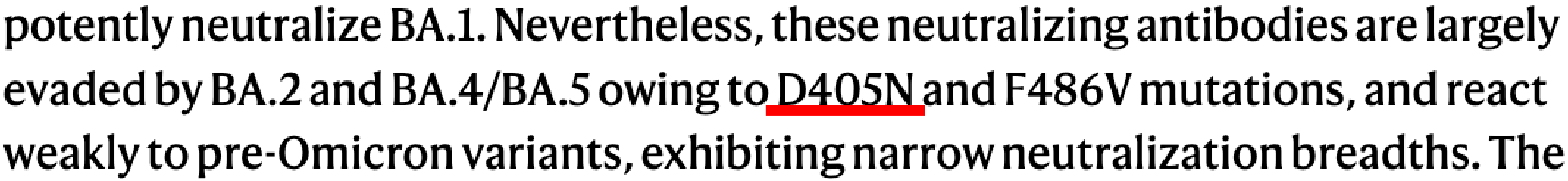
Takeda, Microbiology and Immunology, 2021



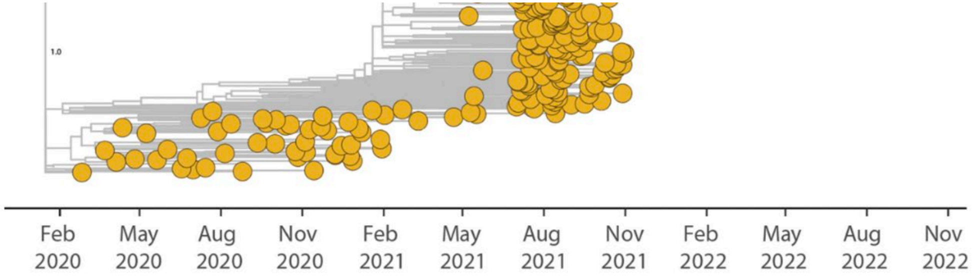
Mutations in the Integrin-Binding RGD Motif of SARS-CoV-2 Variants

Article

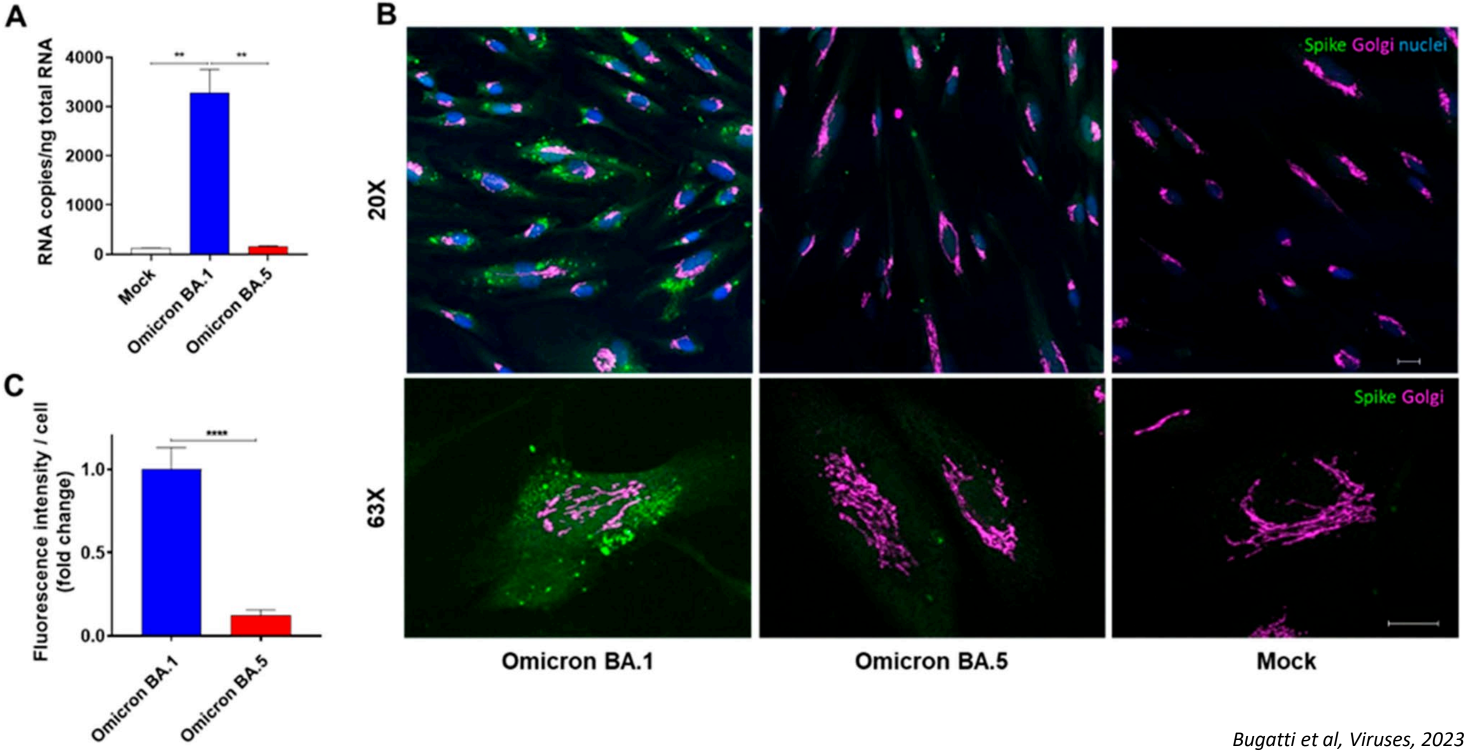
BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection



potently neutralize BA.1. Nevertheless, these neutralizing antibodies are largely evaded by BA.2 and BA.4/BA.5 owing to D405N and F486V mutations, and react weakly to pre-Omicron variants, exhibiting narrow neutralization breadths. The

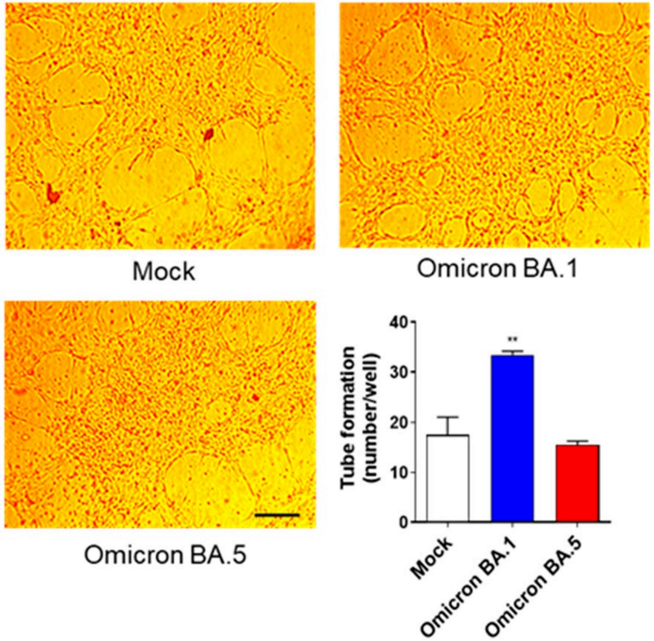


The D405N Mutation in the Spike Protein of SARS-CoV-2 Omicron BA.5 Inhibits Spike/Integrins Interaction and Viral Infection of ECs



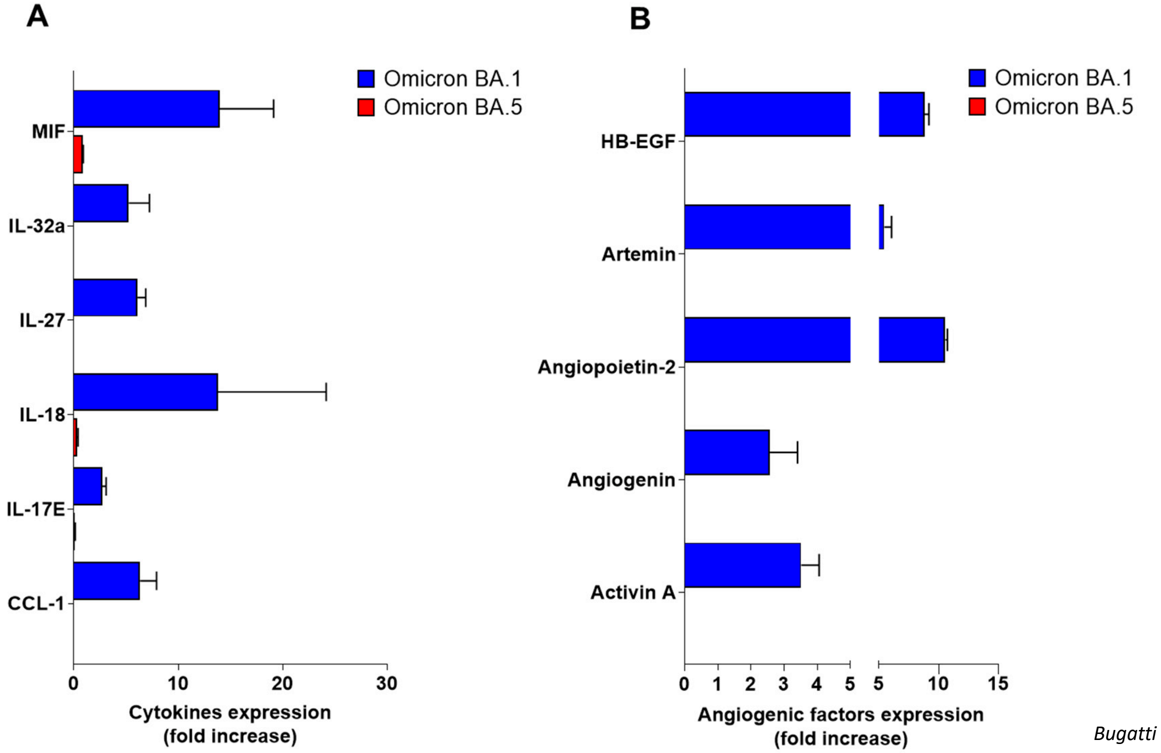
Bugatti et al, Viruses, 2023

SARS-CoV-2 BA.5 Does Not Trigger Angiogenesis



Bugatti et al, Viruses, 2023

SARS-CoV-2 Omicron BA.5 does not induce the Release of inflammatory cytokines and angiogenic molecules from HL-mECs

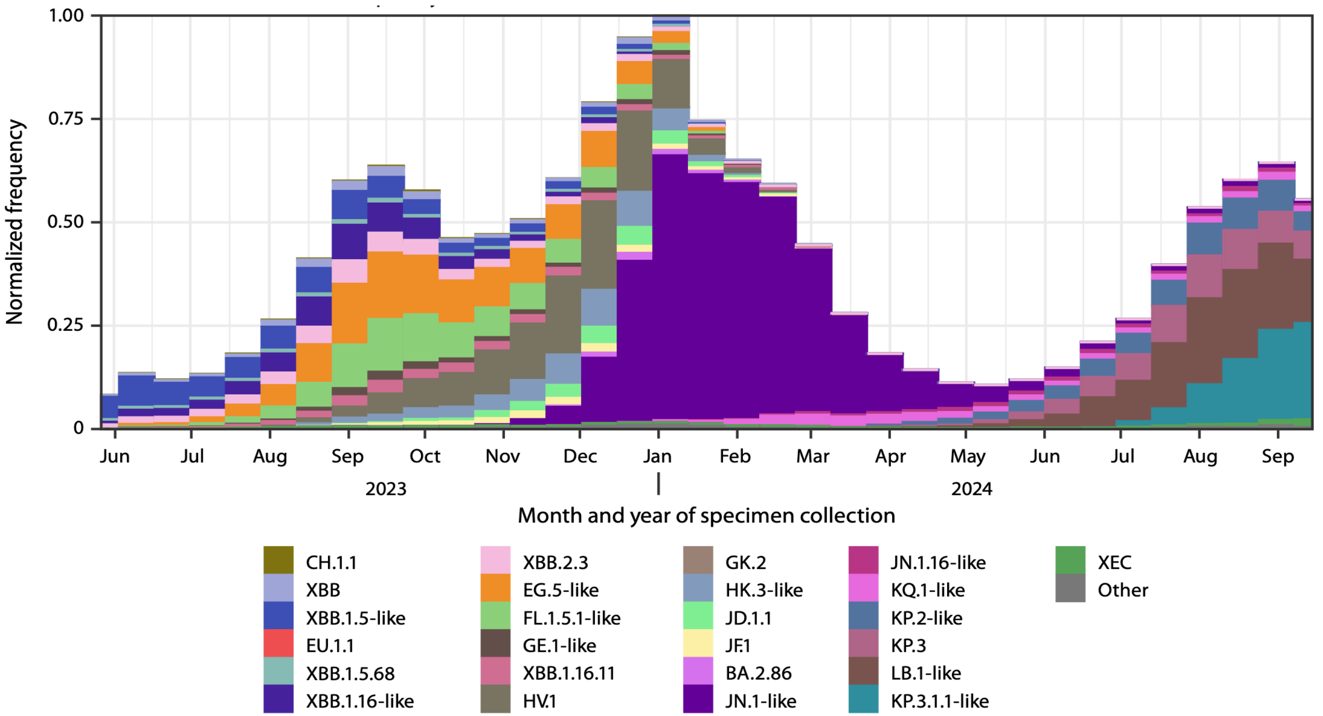


Bugatti et al, Viruses, 2023

Conclusion II

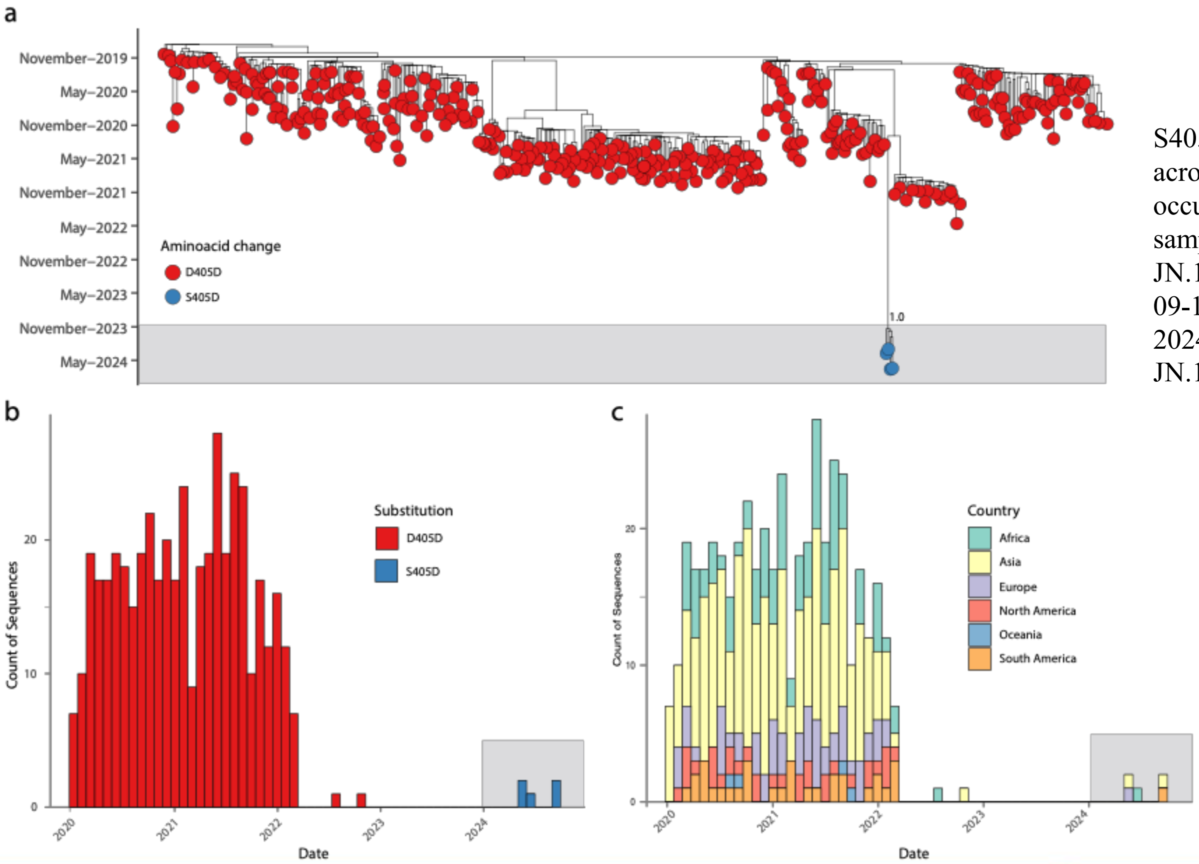
- SARS-CoV-2 enters into ACE2-negative endothelial cells through integrins
- This entry is mediated by an RGD motif on SARS-CoV-2 Spike
- HL-mEC infection promotes the remodeling of cells toward a pro-inflammatory and pro-angiogenic phenotype
- D405N/S mutation induces lack of Spike/integrins interaction inhibiting HL-mECs infection of and dysfunction.

Newly emerged SARS-CoV-2 variants



MMWR, 2024

Homoplasy event?



S405D-carrying sequences were sampled across diverse regions, with specific occurrences in Africa (lineage JN.1.11.1, sampled on 2024-06-19), Asia (lineages JN.1.17 on 2024-05-08 and MB.1.1 on 2024-09-13), Europe (lineage JN.1.11, sampled on 2024-05-27), and South America (lineage JN.1.11, sampled on 2024-09-08).

Conclusion III

- The RGD motif mutated to RGN/RGS under immunological pressure leading to a loss-of-function on endothelial cells (less virulent variants!)
- The back mutation from RGN/RGS to RGD may lead to a dramatic gain-of-function of SARS-CoV-2 promoting endothelialitis
- A continuous genomic surveillance is crucial to deepen our understanding in SARS-CoV-2 evolution and prevent diffusion of new virulent strains

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Thank you for your attention!